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Renal Response to Candoxatrilat in Patients With Heart Failure

JOANNA M. GOOD, MB, MRCP, MICHAEL PETERS, MD, FRCR, MARTIN WILKINS, MD, MRCP, NEVILLE JACKSON, MD, MRCP,* CELIA M. OAKLEY, MD, FRCP, FACC, FESC, JOHN G. F. CLELAND, MD, FRCP, FESC, FACC†

London and Sandwich, Kent, England, United Kingdom

Objectives. **Our primary objective was to compare the effects of three different doses of candoxatrilat with the effects of placebo on urinary volume in patients with moderately severe heart failure. The effects of candoxatrilat on urinary composition, neuroendocrine indexes and renal hemodynamic function were also studied.**

Background. **Candoxatrilat, a neutral endopeptidase inhibitor, reduces degradation of atrial natriuretic peptide and provokes diuresis in patients with mild heart failure, but the renal effects have not been studied in patients with moderately severe heart failure in a placebo-controlled study.**

Methods. **In a double-blind crossover trial, the effects of intravenous boluses of saline vehicle (placebo) and 50, 100 and 200 mg of candoxatrilat were compared on separate days in 12 patients with heart failure. Urinary output and composition were measured for 8 h. Renal blood flow and glomerular filtration rate were determined by radionuclide techniques. Blood was withdrawn for the measurement of hormones before and 3 h after dosing.**

Results. **All doses of candoxatrilat increased urinary volume (e.g., [mean ± SEM] 263 ± 53 to 490 ± 82 ml for saline solution and the 200-mg dose, respectively, $p < 0.01$) and sodium content (14 ± 4 to 37 ± 11 mmol, $p < 0.001$) in the 1st 4 h after dosing. Plasma atrial natriuretic peptide increased (140 ± 26 to 279 ± 37 pg/ml, $p < 0.01$), whereas aldosterone decreased (178 ± 41 to 125 ± 35 pg/ml, $p < 0.01$), and renin activity was unchanged (10 ± 2 to 12 ± 3 ng/ml per h).**

Conclusions. **Candoxatrilat given acutely causes diuresis, even in patients with moderately severe heart failure.**

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The biologic actions of atrial natriuretic peptide include natriuresis-diuresis, vasorelaxation and inhibition of the renin-angiotensin system (1). Plasma levels of atrial natriuretic peptide are elevated in patients with heart failure in proportion to the degree of myocardial dysfunction (2), representing a compensatory response tending to reduce the hemodynamic load.

Therapeutic use of the peptide is limited by its short plasma half-life (2 to 3 min) and its susceptibility to degradation in the gut (1). Another approach is to inhibit the enzymatic degradation of the peptide by neutral endopeptidase EC3.4.24.11 (3), an important clearance pathway for circulating atrial natriuretic peptide. Studies in animals have shown that neutral endopeptidase inhibition enhances and prolongs the natriuretic and hypotensive effects of coinfused exogenous atrial natriuretic peptide (4) and increases sodium excretion in animal models of hypertension and heart failure (5,6).

Given to healthy volunteers and patients with essential hypertension, in whom plasma atrial natriuretic peptide levels are normal or only modestly elevated, neutral endopeptidase inhibitors approximately double sodium excretion (7). A larger natriuresis (up to six-fold) has been reported in hypertensive patients on a high sodium diet (8), a circumstance associated with high levels of atrial natriuretic peptide, and prominent effects might also be anticipated in heart failure (9).

In preliminary studies (10,11) ±candoxatrilat (UK-69,578, the racemic mixture of which candoxatrilat is the active component) and sinorphan have reduced pulmonary capillary wedge pressure and right atrial pressure in patients with mild to moderate heart failure. In addition, Northridge et al. (11) observed a 2.5-fold increase in sodium excretion produced by neutral endopeptidase inhibition in patients with mild heart failure (New York Heart Association functional classes I and II). In a small open study without placebo control, Münzel et al. (12) reported that candoxatrilat produced diuresis and natriuresis in patients with moderately severe heart failure (12). The present study was designed to examine in detail the acute renal response to candoxatrilat, a potent selective inhibitor of neutral endopeptidase, given as a single bolus to patients with moderate to severe heart failure in a double-blind randomized trial.

From the Departments of Medicine (Cardiology) and Nuclear Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London; and *Pfizer Central Research, Sandwich, Kent, England, United Kingdom. Pfizer Central Research provided the trial medication and the financial support for Dr. Good. Dr. Cleland is supported by the British Heart Foundation, London.

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†Present address and address for correspondence: Dr. John G. F. Cleland, Medical Research Council Clinical Research Initiative in Heart Failure, West Medical Building, University Avenue, Glasgow University, Glasgow, Scotland, United Kingdom G12 8QQ.

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Table 1. Patient Characteristics at Baseline

Pt No.	Age (yr)/ Gender	Diagnosis	EF (%)	Daily Dose (mg)		Serum Creatinine (μmol/liter)	NYHA Class
				Furosemide	ACE Inhibitor		
1	48/M	DCM	15	40	15, enalapril	121	III
2	52/M	IHD	24	120	—	88	II
3	64/M	IHD	25	—	37.5, captopril	93	II
4	50/M	IHD	17	40	10, enalapril	148	III
5	56/M	DCM	40	80	5, enalapril	103	II
6	68/M	DCM	23	160	15, enalapril	106	III
7	65/M	DCM	31	120	10, enalapril	175	III
8	59/M	IHD	39	100	25, captopril	118	III
9	57/M	IHD	15	120	—	97	III
10	72/M	IHD	24	80	37.5, captopril	159	II
11	63/M	Mitral regurgitation	25	40	20, lisinopril	138	III
12	54/M	IHD	33	80	—	117	II

ACE = angiotensin-converting enzyme; DCM = dilated cardiomyopathy; EF = left ventricular ejection fraction; IHD = ischemic heart disease; M = male; NYHA Class = New York Heart Association functional class; Pt = patient.

Methods

Patients (Table 1). To enter the study, patients had to have a history of heart failure, be receiving treatment for that indication and have evidence of left ventricular dysfunction as evidenced by an echocardiographic end-diastolic dimension >58 mm and fractional shortening <20% or an ejection fraction <30% on multiple uptake gated acquisition radionuclide ventriculography. Patients had to be in functional class II or III, have a serum creatinine level >200 mmol/liter and B1 sinus rhythm or well controlled atrial fibrillation.

Twelve men, mean age 59 years (range 48 to 72), with chronic heart failure were studied. All patients had a history of prior episodes of pulmonary edema, but their condition had subsequently stabilized with medical therapy. At the time of recruitment into the study, 10 patients gave a recent history of orthopnea, 7 were breathless on moderate exertion and 4 were breathless on slight exertion. Seven patients were in functional class III and five in class II at the time of study. Four patients were awaiting heart transplantation.

Seven patients had sinus rhythm and five had well controlled atrial fibrillation. The mean ± SEM left ventricular ejection fraction was 26 ± 2%. Seven patients were taking both diuretic drugs and an angiotensin-converting enzyme inhibitor; three patients were taking diuretic drugs only. One patient with severe ventricular dysfunction and mitral regurgitation required 80 mg day of furosemide to control heart failure but was able to stop taking diuretic drugs before inclusion in this study, having responded well to the introduction of captopril.

Study protocol. Each patient received in double-blind, random order placebo or candoxatrilat at doses of 50, 100 or 200 mg administered by slow intravenous bolus over 5 min at the same time of day on 4 separate days. Studies were at least 48 h and usually 1 week apart (median 28 days from first to fourth study, range 11 to 63 days). Treatment order was determined by a computer-generated random code and used a balanced latin square design. Each patient served as his own control.

Patients were placed on a diet containing 100 mmol of sodium and 40 mmol of potassium for 3 days before each study day. A dietary history had determined that the usual median sodium intake of the study patients before this experiment was 135 mmol/day. Cardioactive medications were omitted on the morning of each study day. On arrival, the patient emptied the bladder, had standing blood pressure recorded and then remained supine until the end of the study day. Intravenous cannulas were inserted in antecubital veins bilaterally and the patient ate a set breakfast. After the patient had rested for 2 h, venous samples were taken for osmolality and hormone levels and he emptied the bladder again. Blood pressure was recorded and the test drug was administered.

Over the subsequent 8 h urinary samples were obtained every 2 h. Blood pressure was recorded at 1, 2 and 4 h after dosing. Three hours after dosing, the patient underwent radionuclide studies (see later) and a blood sample was taken for hormone assay. Under supervision, the patient was administered 200 ml of fluid in each 2-h period and a standardized light meal shortly after completion of the radionuclide studies. At the end of the study period, supine and standing blood pressure was recorded, and the patient was reexamined and discharged.

Measurements. Heart rate was counted from the radial pulse, or cardiac apex for those with atrial fibrillation, and blood pressure was measured by using the same calibrated mercury sphygmomanometer.

Urinary volume was measured and aliquots taken for measurement of cyclic guanosine monophosphate (GMP), sodium, potassium, magnesium, chloride, calcium, phosphate, urea, creatinine and osmolality. Urinary pH was measured by using Multistix (Ames). Urinary sodium and potassium levels were measured by using a Beckman E2A ISE analyzer; phos-

Table 2. Effects of Candoxatrilat on Urinary Volume, Electrolytes and Cyclic Guanosine Monophosphate

Urinary Variables	Placebo	Candoxatrilat 50 mg	Candoxatrilat 100 mg	Candoxatrilat 200 mg	p Value (pooled comparison)
Volume (ml)					
0–4 h	230 ± 36	471 ± 51*	466 ± 71†	489 ± 82†	< 0.0001
4–8 h	293 ± 46	356 ± 44	397 ± 60	369 ± 49	< 0.01
Sodium (mmol)					
0–4 h	14 ± 4	31 ± 7†	28 ± 7†	37 ± 11‡	< 0.0001
4–8 h	11 ± 2	20 ± 5	23 ± 6†	20 ± 4*	< 0.001
Chloride (mmol)					
0–4 h	12 ± 5	25 ± 6‡	21 ± 6†	30 ± 9‡	< 0.0001
4–8 h	8 ± 3	15 ± 5	16 ± 5*	13 ± 3	< 0.001
Potassium (mmol)					
0–4 h	9 ± 1	12 ± 2*	12 ± 1*	14 ± 2*	< 0.01
4–8 h	9 ± 1	8 ± 1	9 ± 1	9 ± 1	NS
Magnesium (mmol)					
0–4 h	0.5 ± 0.1	0.8 ± 0.1†	0.8 ± 0.1†	0.8 ± 0.1†	< 0.0001
4–8 h	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	NS
Bicarbonate (mmol)					
0–4 h	1.6 ± 0.5	5.0 ± 1.7†	6.0 ± 2.6†	5.0 ± 1.8‡	< 0.0001
4–8 h	1.4 ± 0.5	2.6 ± 0.7	3.9 ± 1.5*	3.0 ± 0.9	< 0.0001
Calcium (mmol)					
0–4 h	0.6 ± 0.1	1.1 ± 0.2†	1.3 ± 0.2†	1.1 ± 0.2†	< 0.0001
4–8 h	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	<0.01
Phosphate (mmol)					
0–4 h	3.5 ± 0.2	4.3 ± 0.7	4.3 ± 0.7*	4.3 ± 0.5*	< 0.01
4–8 h	4.3 ± 0.4	3.3 ± 0.4	4.7 ± 0.5	4.2 ± 0.4	NS
Urea (mmol)					
0–4 h	63 ± 7	82 ± 11*	84 ± 9*	81 ± 9*	< 0.001
4–8 h	68 ± 8	63 ± 7	74 ± 7	69 ± 6	NS
Creatinine (μmol)					
0–4 h	1.7 ± 0.1	1.9 ± 0.2*	1.9 ± 0.1*	2.0 ± 0.2*	< 0.001
4–8 h	2.0 ± 0.1	1.7 ± 0.2	2.0 ± 0.1	1.9 ± 0.1	NS
Cyclic GMP (pmol)					
0–4 h	26 ± 5	82 ± 15*	99 ± 19†	99 ± 22†	< 0.0001
4–8 h	31 ± 6	57 ± 11	80 ± 11†	91 ± 13†	< 0.0001

*, †, ‡ indicate differences of individual data points from values with placebo: *p < 0.05, †p < 0.01, ‡p < 0.001. Data are expressed as mean value ± 1 SEM. GMP = guanosine monophosphate.

phate, urea and creatinine by using the SMAC-2 analyzer (Technicon), and magnesium by using a Technicon RA-XT analyzer with proprietary agents. Chloride was measured on a CCMI chloride meter (Chemlab Sci Equipt) with Chemlab proprietary reagents. Osmolality was measured with a Clandon Scientific Osmomat. Urinary cyclic GMP was assayed by a double-antibody radioimmunoassay (Biomedical Technologies Inc.).

Venous blood samples were drawn from the antecubital fossa on each day immediately before administration of candoxatrilat and 3 h after dosing at the expected time of maximal effect. These samples were later assayed as previously described (2,13,14) for plasma renin activity, aldosterone, atrial natriuretic peptide and vasoactive intestinal polypeptide. In addition, 2-hourly samples were analyzed for plasma osmolality.

Cardiac output was measured by standard technique (15) and renal blood flow was measured by using a first-pass technique from a bolus of 500 mBq of technetium-99m–labeled albumin (16). Renal blood flow to each kidney as a fraction of cardiac output was measured from the same first-pass dynamic data (16–18). Owing to the poor left ventricular function in these patients and a resulting wide bolus (19), the ratio of renal blood flow to cardiac output was based on a region of interest placed over the left ventricle and corrected for the lung signal from the base of the left lung (16). Total renal blood flow, expressed either as a percent of cardiac output or in absolute terms, was obtained by summing the values for the left and right kidneys. Renal vascular resistance was calculated from the renal blood flow and the mean arterial pressure. Left ventricular ejection fraction was obtained from the equilibrium phase of the injection with the use of gated acquisition over a minimum of 600 cardiac cycles within a defined representative RR interval on each occasion. Glomerular filtration rate was derived from the clearance of 51-chromium ethylenediaminetetraacetic acid (20).

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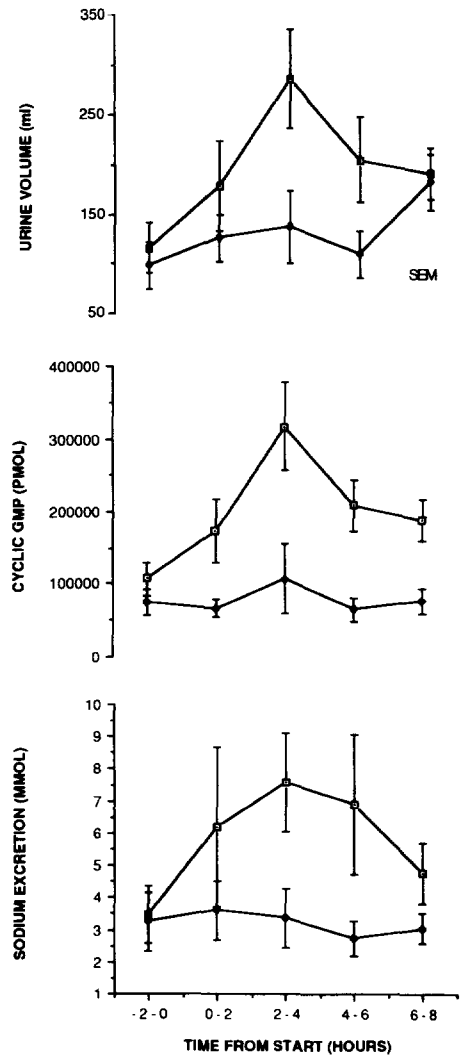

Figure 1. Plot of change in urinary volume, urinary cyclic guanosine monophosphate (GMP) and urinary sodium content over time after administration of placebo (**circles**) and candoxatrilat, 100 mg (**squares**). Variables are expressed as mean value ± 1 SE.

Statistical analysis. The sample size of 12 patients was chosen as sufficient to detect a mean difference of 800 ml in cumulative urinary output (over 8 h) between placebo and any of the active treatment groups at the 5% level of significance with a p value >0.8. This calculation was based on the assumption that the within-patient standard deviation for urinary output on repeat occasions would be 798 ml in the absence of medication. In the light of the results of the study, the within-patient standard deviation for urinary output was a gross overestimate; therefore, the study had the power to detect considerably smaller differences in urinary volume.

Analysis of variance testing was applied, within-patient, for period, treatment and carryover effects. The treatment sums of squares were partitioned into orthogonal components due to 1) pooled candoxatrilat versus placebo; 2) a linear dose-response relation for candoxatrilat; 3) a quadrantic dose response for candoxatrilat. The Dunnett statistic (21) was used, with values < 0.05 being considered significant. Values are presented as mean value ± SEM. The primary statistical analysis compared the pooled results of active therapy (all three doses of candoxatrilat) versus placebo only when analysis of variance had already indicated a treatment difference and that no difference among the effects of the three doses of candoxatrilat was present. Data for individual doses compared with placebo after correction for multiple comparisons are also shown.

Results

No significant differences were noted among the three doses of candoxatrilat for any measured variable. No effect of the order of treatment was noted.

Urinary volume (Table 2, Fig. 1). Compared with placebo, candoxatrilat increased urinary volume over the 8 h. The diuresis occurred predominantly over the 1st 4 h, although effects persisted for at least 6 h after dosing. There was no relation between the dose of candoxatrilat and the urinary response. Oral fluid was administered at 100 ml/h on each study day (see Methods). A cumulative positive fluid balance occurred during the placebo period, whereas fluid balance was negative during candoxatrilat study days.

Urinary composition (Tables 2 and 3). Urinary sodium content increased, with the most striking effects observed in the 1st 4 h after candoxatrilat administration, but an effect was sustained over the entire 8-h observation period. Urinary chloride excretion paralleled that of sodium. Urinary potassium increased in the 1st 4-h period, but the effect was not sustained. The excretion of potassium was not significantly increased over the 8 h of study.

Candoxatrilat rendered the urine more alkaline than did placebo. Urinary magnesium, bicarbonate and calcium increased after candoxatrilat administration, especially in the 1st 4 h. Urinary phosphate, urea and creatinine increased only in the 1st 4-h collection period. Urinary osmolality decreased, reflecting a decline in urinary potassium, magnesium, phosphate, urea and creatinine concentration. The urinary concentration of sodium, chloride and bicarbonate increased.

The ratio of sodium, potassium, magnesium and bicarbonate to creatinine all increased in the 1st 4 h, reflecting increased fractional excretion of these electrolytes. The potassium/creatinine ratio was similar during placebo and active therapy during the 2nd 4-h period, but the ratio for other electrolytes remained elevated. The ratio of phosphate to creatinine was unchanged throughout the study, whereas the ratio of sodium to phosphate increased.

Urinary cyclic GMP (Table 2). Urinary cyclic GMP increased markedly after each dose of candoxatrilat, but no significant difference between doses was observed.

Hormones (Table 4). Plasma concentrations of atrial natriuretic peptide ($p < 0.001$) and vasoactive intestinal polypeptide ($p = 0.01$) increased, whereas those of aldosterone ($p < 0.01$) were reduced. Plasma renin activity did not change. These effects were not dose related.

Table 3. Effects of Candoxatrilat on Urinary Osmolality and Excretion of Electrolytes

	Placebo	Candoxatrilat 50 mg	Candoxatrilat 100 mg	Candoxatrilat 200 mg	p Value (pooled comparison)
Osmolality (mOsm/kg)					
0–4 h	992 ± 101	738 ± 93†	745 ± 69†	777 ± 75*	< 0.001
4–8 h	937 ± 108	641 ± 77†	783 ± 70*	823 ± 86	< 0.05
Sodium/creatinine (mmol/μmol)					
0–4 h	8 ± 2	18 ± 4†	16 ± 5†	19 ± 6†	< 0.0001
4–8 h	6 ± 1	12 ± 2†	11 ± 3*	11 ± 2*	< 0.0001
Potassium/creatinine (mmol/μmol)					
0–4 h	5.1 ± 0.4	6.2 ± 0.6	6.3 ± 0.6*	7.0 ± 1.0†	< 0.001
4–8 h	4.7 ± 0.6	4.7 ± 0.5	4.3 ± 0.5	5.0 ± 0.6	NS
Magnesium/creatinine ([mmol × 100]/μmol)					
0–4 h	30 ± 4	41 ± 2†	42 ± 4†	43 ± 3†	< 0.0001
4–8 h	24 ± 5	34 ± 4*	33 ± 1*	34 ± 4*	< 0.01
Bicarbonate/creatinine (mmol/μmol)					
0–4 h	0.9 ± 0.3	2.5 ± 0.7†	3.0 ± 1.1†	2.4 ± 0.7†	< 0.0001
4–8 h	0.7 ± 0.2	1.8 ± 0.5	1.8 ± 0.6	1.5 ± 0.4	< 0.01
Phosphate/creatinine (mmol/μmol)					
0–4 h	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	NS
4–8 h	2.3 ± 0.1	2.1 ± 0.2	2.2 ± 0.2	2.4 ± 0.3	NS

*, † indicate differences of individual data points from values with placebo: *p < 0.05, †p < 0.01. Data are expressed as mean value ± 1 SEM.

Heart rate and blood pressure (Table 5). Heart rate recorded after administration of each dose of candoxatrilat did not differ from the rate recorded after placebo administration at any time. No significant change in blood pressure in either the supine or the standing position was observed after any dose of candoxatrilat.

Ejection fraction and renal hemodynamic variables (Table 6). Left ventricular ejection fraction and cardiac output were unchanged 3 h after candoxatrilat administration. Renal blood flow and glomerular filtration rate tended to increase with candoxatrilat and renal vascular resistance tended to decrease (p = NS). Renal blood flow expressed as a percent of cardiac output did increase.

Discussion

To our knowledge this is the first comprehensive study of the acute renal response to neutral endopeptidase inhibition in patients with moderate to severe heart failure. Candoxatrilat produced a substantial increase in sodium and water excretion. Despite the diuresis, plasma atrial natriuretic peptide increased, there was no activation of the renin-angiotensin system and aldosterone decreased. Each dose of candoxatrilat provoked a similar diuresis and increase in plasma atrial natriuretic peptide, suggesting that all doses exceeded the minimal inhibitory concentration for neutral endopeptidase.

Brief review of the effects of atrial natriuretic peptide in health and heart failure. The response to intravenous atrial natriuretic peptide in healthy subjects includes increases in urinary volume and sodium, phosphate, calcium, magnesium and chloride excretion (22). Reports of an increase in urinary potassium excretion have been inconsistent (9,23). An increase in hematocrit during infusion of atrial natriuretic peptide suggests a shift of fluid out of the intravascular compartment (9,24) that may, in part, be responsible for the decrease in cardiac filling pressures (22). Changes in cardiac output and

Table 4. Effects of Candoxatrilat on Neuroendocrine Variables

	Placebo	Candoxatrilat 50 mg	Candoxatrilat 100 mg	Candoxatrilat 200 mg	p Value (pooled comparison)
ANP (pg/ml)	140 ± 26	263 ± 37†	320 ± 65†	279 ± 37†	< 0.001
Plasma renin activity (ng/ml per h)	10.1 ± 2.1	7.3 ± 1.9	9.2 ± 2.1	11.9 ± 2.6	NS
Aldosterone (pg/ml)	178 ± 41	108 ± 24*	103 ± 26*	125 ± 35†	< 0.01
VIP (pmol/liter)	8.0 ± 1.7	12.2 ± 1.5	13.4 ± 1.7*	11.5 ± 2.0	0.01

*, † indicate differences of individual data points from values with placebo: *p < 0.05, †p < 0.01. Data are expressed as mean value ± 1 SEM. ANP = atrial natriuretic peptide; VIP = vasoactive intestinal polypeptide.

Table 5. Effects of Candoxatrilat on Heart Rate and Blood Pressure

	Placebo	Candoxatrilat 50 mg	Candoxatrilat 100 mg	Candoxatrilat 200 mg
Heart rate (beats/min)				
4 h	76 ± 4	76 ± 4	74 ± 4	76 ± 4
8 h	78 ± 4	77 ± 3	77 ± 4	78 ± 4
Supine BP (mm Hg)				
Systolic				
4 h	114 ± 3	113 ± 4	112 ± 4	108 ± 4
8 h	113 ± 3	113 ± 3	111 ± 6	110 ± 3
Diastolic				
4 h	74 ± 2	71 ± 2	69 ± 2	66 ± 3
8 h	77 ± 2	74 ± 3	70 ± 3	70 ± 2
Standing BP (mm Hg)				
Systolic				
8 h	115 ± 6	110 ± 6	106 ± 5	107 ± 6
Diastolic				
8 h	74 ± 3	72 ± 3	69 ± 3	68 ± 3

Data are expressed as mean value ± 1 SEM. There were no significant differences from values with placebo. BP = blood pressure.

systemic vascular resistance in response to atrial natriuretic peptide in normal subjects are small (9,23).

In patients with heart failure the increase in urinary volume and natriuresis elicited by atrial natriuretic peptide is blunted (9) and kaliuresis is observed (23,25). Hematocrit does not increase during infusions of atrial natriuretic peptide for up to 1 h in patients with heart failure (9,25), although during longer infusions it may increase (26), and in a previous study of heart failure candoxatrilat did not increase hematocrit (12). Unlike the urinary response, the vascular response to atrial natriuretic peptide is not grossly impaired in heart failure. Atrial natriuretic peptide reduces systemic vascular resistance and filling pressures and increases cardiac output (9,23). As hematocrit does not increase, a venodilator effect of atrial natriuretic peptide, possibly by inhibiting angiotensin II (27) or sympathetically mediated venous constriction (28,29), seems likely to account for the reduction in filling pressures.

The reason for the attenuated renal response to atrial natriuretic peptide in human heart failure is not known. Among the mechanisms proposed are down-regulation of atrial natriuretic peptide receptors (30), changes in renal perfusion pressure (31,32) or intrarenal hemodynamic variables and increased sympathetic activity (33,34). Alternatively, intraluminal degradation of atrial natriuretic peptide in the renal tubule may be increased as a result of either enzyme induction or a longer tubular transit time allowing prolonged exposure and degradation of atrial natriuretic peptide before it can reach a more distal site of action.

Potential mechanisms of the renal effects of candoxatrilat. Although atrial natriuretic peptide antibodies abolish the renal response to candoxatril (35), atrial natriuretic peptide may not be the sole mediator of the renal response. Neutral endopeptidase is capable of metabolizing a number of other peptides including bradykinin (37), brain natriuretic peptide (38), and, from the present study, vasoactive intestinal peptide. Interestingly, the renal response to brain natriuretic peptide, unlike that to atrial natriuretic peptide, may not be impaired in heart failure (38–41). Bradykinin and vasoactive intestinal peptide are also capable of stimulating a natriuresis (37,42).

Filtered atrial natriuretic peptide and brain natriuretic peptide may exert their effects on the inner medulla on their passage through the nephron rather than in the circulation. Receptors for atrial natriuretic peptide are found in the inner medullary collecting duct, whereas neutral endopeptidase is found in abundance in the brush border of the proximal tubule (43,44). Animal studies show that NEP inhibition protects atrial natriuretic peptide from degradation in the kidney and leads to the excretion of increased amounts of immunoreactive atrial natriuretic peptide (45). However, an increase in urinary atrial natriuretic peptide after candoxatrilat administration has not been demonstrated in humans.

Changes in urinary composition lend some insights into the renal effects of candoxatrilat. Urinary creatinine increased initially after administration of candoxatrilat, probably reflecting an increase in glomerular filtration rate, although this was not borne out by the radioisotope measurements of glomerular filtration rate. The ratio phosphate/creatinine, an index of reabsorption from the second half of the proximal tubule (46), was unaltered. Urinary potassium initially increased, as has been observed with atrial natriuretic peptide (23,25), but this increase did not persist, possibly owing to the suppression of plasma aldosterone. Magnesium and bicarbonate are reabsorbed in both the proximal and the distal nephron (47), and

Table 6. Hemodynamic Effects of Candoxatrilat at 3 Hours After Administration

	Placebo	Candoxatrilat 50 mg	Candoxatrilat 100 mg	Candoxatrilat 200 mg
GFR (ml/min)	72.0 ± 8.2	78.3 ± 9.3	89.3 ± 8.2	76.0 ± 6.8
RPF (ml/min)	298 ± 50	378 ± 65	379 ± 64	305 ± 25
RBF/CO (dynes·s·cm^{-5})	10.1 ± 1.0	12.6 ± 1.5	10.3 ± 4.4	11.3 ± 1.2 (p = 0.053*)
RVR (dynes·s·cm^{-5})	0.36 ± 0.05	0.27 ± 0.03	0.31 ± 0.06	0.29 ± 0.03
CO (liters/min)	3.0 ± 0.2	3.0 ± 0.2	3.5 ± 0.2	2.7 ± 0.2

*p value comparing placebo and pooled active therapy. There were no statistically significant differences between individual or pooled data points comparing placebo and candoxatrilat therapy. Data are expressed as mean value ± 1 SEM. CO = cardiac output; GFR = glomerular filtration rate; RBF = renal blood flow; RPF = renal plasma flow; RVR = renal vascular resistance.

Table 7. Heterogeneity of Urinary Volume in Response to Candoxatrilat (0 to 4 h)

Pt No.	Placebo	Candoxatrilat 50 mg (ml)	Candoxatrilat 100 mg (ml)	Candoxatrilat 200 mg (ml)	Average Active Treatment (ml)	Average Increase With Candoxatrilat* ml	Average Increase With Candoxatrilat* %
1	184	360	465	320	382	198	107
2	124	312	352	243	302	178	144
3	298	574	574	724	632	334	112
4	534	900	1,038	1,199	1,046	512	96
5	309	465	365	508	446	137	44
6	176	541	824	722	696	520	295
7	343	454	284	375	371	28	8
8	210	605	412	430	482	272	130
9	141	213	244	236	231	90	64
10	215	401	267	264	311	96	44
11	110	385	516	273	391	281	256
12	115	430	250	569	416	301	262

*Average active treatment value minus placebo value. Pt = patient.

urinary content and fractional excretion, at least of the latter, were increased throughout the study. Overall, these data suggest that the initial increase in sodium excretion is due to a combination of increased filtered load and effects distal to the proximal renal tubule. Later, the filtered load appears to return to normal, but distal tubular reabsorption of sodium in exchange for potassium is reduced secondary to effects on aldosterone.

The present study failed to show an effect of candoxatrilat on renal blood flow. The technical variability in measuring absolute cardiac output and renal blood flow by our method may be large among patients with poor ventricular function, although comparisons with simultaneous thermodilution measurements have been favorable even in such patients (48). The hemodynamic measurements were obtained over a short time 3 h after the injection of candoxatrilat; we cannot say what blood flow measurements might have been at other times. Our results do not rule out an effect on renal blood flow and, indeed, when this flow is expressed as a percent of cardiac output, the data suggest that it is increased after candoxatrilat.

The reasons why some patients responded better than others to candoxatrilat are uncertain from the present study. No baseline variable, treatment regime, neuroendocrine or renal hemodynamic response was clearly related to the magnitude of response (Tables 1 and 7). Further studies to determine which factors are responsible for the heterogeneity in response are needed.

Potential advantages of neutral endopeptidase inhibitors over conventional diuretic agents. Until the last decade, conventional diuretic agents were the only generally accepted highly effective agents for the management of congestive heart failure. These agents lessen the symptoms and signs of heart failure (49) and are essential for the management of acute heart failure, but there are no trials to test their impact on prognosis. Conventional diuretic agents are attended by many side effects such as diabetes and gout, and activation of the renin-angiotensin system. Conventional diuretic therapy reduces plasma atrial natriuretic peptide, impairing a compensatory mechanism resisting sodium retention (50–52). The neuroendocrine effects associated with conventional diuretic drugs may reduce their long-term effectiveness, lead to acceleration in the decline of ventricular function and increase the incidence of arrhythmias and sudden death. A diuretic agent such as candoxatrilat that simulated the actions of atrial natriuretic peptide, provoking a diuresis and simultaneous inhibition of neuroendocrine activation, could have considerable therapeutic potential for the treatment of heart failure.

Limitations of the present study. There is little experience as yet with neutral endopeptidase inhibitors in human heart failure. At the time of inception of the study we believed that the doses we had chosen would span the therapeutic range of the drug; however, all the doses we used appeared to be at the top of the dose-response curve. We may also be criticized for not measuring cardiac and renal hemodynamics directly by invasive means. We considered this impossible given the study design. Moreover the primary objective of the study was to assess the effects of candoxatrilat on urinary volume.

We cannot say what the magnitude of the urinary response to candoxatrilat is in patients with heart failure compared with that of normal subjects (53), although in this study it was similar to that observed in mild heart failure (11). Studies in rats suggest that heart failure may augment the diuretic effects of candoxatrilat, an effect that was attributed to higher levels of atrial natriuretic peptide (54). These results must be extrapolated with caution to human heart failure; the effect of candoxatrilat may be augmented in untreated human heart failure but long-term diuretic therapy and possibly angiotensin-converting enzyme inhibitors may reduce this effect by decreasing atrial natriuretic peptide (55).

Treatment before the study was heterogeneous, a factor that may have influenced the results, although we did not observe such effects. However, each patient acted as his own control and withheld his usual medication similarly on each study day. Even if the effects of angiotensin-converting enzyme

inhibition had persisted into the study, they would not have invalidated these placebo-controlled results.

Conclusions. Candoxatrilat causes a diuresis in patients with moderately severe heart failure, increasing atrial natriuretic peptide and vasoactive intestinal polypeptide and suppressing aldosterone without activating the renin-angiotensin system. The mechanism of the changes in salt and water excretion remains to be fully elucidated, but the present study suggests that the predominant effects are an increase in the filtered load of sodium and on the nephron distal to the proximal tubule. There is an as yet unexplained heterogeneous response among patients. The effects of candoxatrilat in heart failure are worthy of further investigation.

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